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STUDIES ON *CERCOSPORA INDICA*, N. SP., PARASITIC ON *CAJANUS INDICUS* SPRENG.

BY

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(With Plates XXI-XXIII and four text-figures)

CONTENTS

I. Introduction.

A leaf-spot disease of *Cajanus indicus* caused by a species of *Cercospora* is very common in Bihar and the United Provinces, and differs in morphology

from *C. Cajani* Rangel and P. Henn. and *C. instabilis* Rangel previously recorded on this host from other countries, *i.e.*, St. Rio de Janeiro. Sio Paulo, Minas, Geraes. Brasiliae and Niteroy. Brasiliae respectively. Two strains of this species were isolated, one (CA)* from Allahabad and the other (CP)* from Pusa and were found to be strains of a new species hitherto undescribed. In addition to this, *C. dolichi* E. and E. which causes infection of *Dolichos Lablab* Linn. was also noticed to infect *Cajanus indicus*, especially when the two crops are grown side by side.

The present study deals with the parasitism of these two strains. their cultural characteristics on different artificial media, and their physiological response to various environmental conditions.

II. Morphology of the organism in nature.

The disease makes its first appearance on the under-surface of leaves as small light brown spots, 1-2 mm. in diameter. These spots are at first more or less roundish, but later on become irregular in outline, and occasionally several coalesce forming irregular areas as large as 15 mm. $\times$ 5 mm. Spots seldom, if ever, cross the midrib or the primary veins of the leaflets. The centre of these spots is dark brown and bear the fascile of conidiophores with conidia (Plate XXI, figs. 1-6). On older spots where conidiophores have ceased to form spores, infected areas become very thin and translucent. In advanced stages the whole of the leaf dries, curls and ultimately falls. The lesions on petioles are less common than on leaves, but more than on stems (Plate XXI figs. 7 and 8). These are greyish black and run parallel to the long axis of the petiole.

The mycelium of the fungus is both inter and intra-cellular, the hyphae collect in the air spaces under the stomata and form stromatic masses giving rise to conidiophores which emerge from the stoma. The fungus also forms creeping external mycelium on the surface of the leaf.

Conidiophores are light brown when young, dark brown when mature, and are found mostly on the under-surface of the leaf and come out in definite fascile arising from loose stromatic masses, found in the air spaces of the leaf (Plate XXI. figs. 1-3). Often they are covered with a series of geneculations marking the points of attachment with conidia (Plate XXII, figs. 4-6). Branched conidiophores are by no means rare. These vary in length and septation according to moisture conditions. Their size ranges from 28·0μ-168·0μ $\times$ 3·4μ-7·0μ and septation 2-13 with a slight constriction in some conidiophores just near the septa. Conidiophores or their pieces readily germinate in tap water in 10-12 hours at 25°C. The contents are highly granular due to the presence of refractive oil globules.

* For simplicity these strains will be referred to in the paper by these symbols.

Conidia are hyaline to slightly greenish yellow in colour, multiseptate, abruptly obclavate sometimes vormiform, with indistinct scars less than 2μ in diameter at the distal end (Plate XXII figs. 7-19). Constrictions near septa are very common (Plate XXII, figs. 8 and 9). In (CP) the size varies from 6·8μ-129·0μ ✕ 3·4μ-5·1μ; septation 0-9, average being 38·7μ ✕ 4·2μ; septation mode 2. In strain (CA), the size varies from 6·8μ-108μ ✕ 3·4μ-5·1μ; septation 0-9, average being 36·8μ ✕ 4·2μ; septation mode 2. From above it is clear that both strains are identical in their morphology. The cell conterts of young conidia are perfectly hyaline but in mature spores it is light green and the contents are granular with number of oil globules. In tap water these germinate between 8-10 hours at 30°C. (Plate XXII, figs. 20 and 22). All the cells of the multiseptate conidia do not lose viability when subjected to desiccation. In some conidia, some cells appear to be entirely empty and shrunken while in others the finely granular protoplasm is present. Conidia does not remain viable for a long period as no conidia germinate after about two months. Klotz [1923] found that conidia of *Cercospora apii* Fres. germinated after 170 days of drying; while Lehman [1928] observed that conidia from preserved specimen of *C. diazu* Maru germinated after 79 days. The conidia attained their maximum length under high humidity. The cells of the distal end of the conidia are longer than those at the proximal end. Conidia produced in culture are more hyaline than in nature.

III. Parasitism.

Both the strains (CA) and (CP) are unable to infect *Cajanus indicus* leaves when inoculated with mycelium alone. No infection takes place even when the mycelium is placed on nutrient drops containing 5 per cent. cane sugar *plus* 2 per cent. asparagin or on drops of stale filtrate in which the fungus was allowed to grow for 4, 8, 16 and 32 days.

When spore suspension is sprayed the incubation period is ten days in the case of mature leaves, and fifteen days in the case of immature leaves. The fungus was isolated from artificially infected leaves and single spore cultures obtained and when compared with the original, they proved to be identical. Infection of petioles and stems when inoculated also took infection.

Disinfected seeds were kept in potato-tubes containing sterilized Knops solution and when the seedlings were six days old, they were smeared with spore suspension and kept at 10°C., 20°C., 27·5°C., 30°C. and 35°C. Triplicate tubes were placed in each temperature. No infection took place below 20°C., and above 32·5°C., good infection at 20°C. and 25°C., moderate at 27·5°C. and slight at 30°C. and 32·5°C. Symptoms of the disease appeared after thirteen days and in the advanced stage leaves curled and became yellowish.

Infection takes place readily both in darkness and in light. The observations made agree with those of Klotz [1923] on *Cercospora apii*, Fres., but not with those of McKay and Pool [1918] on *Cercospora beticola* Sacc. on sugar beet who reported that infection probably took place only in the daytime.

The fungus when inoculated on *Dolichos Lablab*, *Glycine hispida*, Maxim, *Phaseolus acontifolius*, Jacq., *Phaseolus radiatus*, Linn., *Phaseolus mungo* Linn. *var Roxburghii* and *Vigna catjang* Endl., failed to infect.

IV. Cultural studies of the fungus.

(A) Macroscopic characters.

(*i*) *Growth on culture media.*—Both the strains when cultivated on a large number of culture media, showed remarkable differences in cultural characters. They differed from each other in (*a*) amount and colour of the aerial mycelium, (*b*) rate of linear growth, (*c*) colour and nature of the submerged mycelium, (*d*) colour of the substratum. A comparative statement of the two strains is given in Table I.

Table I.

Media	Strain (CA)	Strain (CP)
Coons' agar	*Aerial mycelium* abundant, cottony, light lilacy white; edge light greyish indigo and woolly. *Submerged mycelium*, bluish black with dark brown chlamydospores. *Substratum* light titamouse blue; edge light stone colour.	*Aerial mycelium* sparse, cottony, light purplish tinted white with patches of bright greenish grey mycelium. *Submerged mycelium* highly tortuous, dark brown with numerous chlamydospores. *Substratum*, dark forget-me-not blue, edge light horizon blue.
Richards' solution agar	*Aerial mycelium*, profuse, woolly, light sky coloured white, at places light paynes grey. *Submerged mycelium*, highly tortuous greenish brown with abundant chlamydospores. *Substratum*, dark cypress green.	*Aerial mycelium*, scanty, woolly, dark slate grey, edge light mouse colour. *Submerged mycelium*, dark cypress green. *Substratum*, dark ivy green, edge cypress green.
Oatmeal agar	*Aerial mycelium*, copious, cottony, light purplish tinted white, at places light paynes grey. *Submerged mycelium*, bluish black with abundant dark brown chlamydospores. *Substratum*, centre dull greenish grey; edge light plata indigo.	*Aerial mycelium*, scanty, woolly, light paynes grey. *Submerged mycelium*, bluish black with plenty of greenish brown chlamydospores. *Substratum*, dark bluish black.

TABLE I.—*contd.*

Media	Strain (CA)	Strain (CP)
Browns' synthetic agar	*Aerial mycelium*, profuse, cottony, light lilacy white. Growth very poor. *Submerged mycelium*, compact and bluish black. *Substratum*, greenish blue.	*Aerial mycelium*, very sparse cottony pavnes grey. *Submerged mycelium*, light bluish green with sparse chlamydospores. *Substratum*, light cobalt blue.
Prune juice agar . .	*Aerial mycelium*, abundant loose, cottony, greyish white. *Submerged mycelium*, greenish grey with sparse chlamydospores. *Substratum*, light greyish indigo.	*Aerial mycelium*, moderate, woolly, light grey green. *Submerged mycelium*, dark brown, highly tortuous with abundant chlamydospores. *Substratum*, light blue carbonate of copper.
Beyrincks' agar . .	*Aerial mycelium*, sparse cottony, light purplish tinted white, spreading growth. *Submerged mycelium*, light greenish grey with no chlamydospores. *Substratum*, light flesny white.	*Aerial mycelium*, absent. Growth tree-like branching profusely. *Submerged mycelium*, dark listre green. *Substratum*, light golden bronze green.
Cajanus indicus stem .	*Aerial mycelium*, abundant cottony, light purplish tinted white. *Sclerotial bodies*, abundant.	*Aerial mycelium*, less abundant than (CA), cottony, light mouse colour. *Sclerotial bodies*, abundant.
Wheat straw . .	*Aerial mycelium*, scanty cottony, light lilacy white. *Sclerotial bodies*, abundant.	*Aerial mycelium*, scanty, grey. *Sclerotial bodies*, abundant.

Both the strains were also grown on Doxs' agar, Hopkins' agar, Brown's starch medium, *rahar* leaf decoction agar, and plain agar and showed marked differences on cultural characters.

(*ii*) *Influence of depth of media.*—The effect of depth on medium upon linear rate of growth of the two strains was made on Coons' agar and Oatmeal agar at 27·5 C. and the results obtained were similar to those of *Cercospora dolichi* [Singh, 1933], *i.e.*, there is increase in the linear rate of growth with increase in the amount of medium. Similar results were obtained by Mitra [1931] on *Helminthosporium* species.

(*iii*) *Light relations.*—Effect of alternate light and darkness, continuous light from 100 watt electric lamp and continuous darkness, on linear rate of growth of both the strains was carried out and the strains were also grown on liquid solution (Coons'). It was found that the rate of linear growth is greater in alternate light and darkness, less in continuous darkness and least in continuous light. The retarding effect of continuous darkness and continuous light becomes more evident with time. Similar results were obtained with *C. dolichi* E. and E. [Singh, 1933].

(*iv*) ***Relative humidity.***—The effect of different relative humidity on growth of these two strains was carried out and the method followed was similar as in *C. dolichi* by Singh [1933] and the results are given below in Table II.

TABLE II.

Relative humidity	Strains	15 days	25 days	35 days
47	(CA)	9·6 mm.	11·6 mm.	12·5 mm.
	(CP)	5·3 „	6·0 „	8·4 „
68	(CA)	12·6 mm.	14·9 mm.	20·6 mm.
	(CP)	10·5 „	10·6 „	19·0 „
70·4	(CA)	13·0 mm.	15·6 mm.	22·0 mm.
	(CP)	11·0 „	11·5 „	20·0 „
78·7	(CA)	13·2 mm.	16·0 mm.	27·2 mm.
	(CP)	11·3 „	12·0 „	24·4 „
92·3	(CA)	13·5 mm.	20·5 mm.	31·2 mm.
	(CP)	11·3 „	12·3 „	26·4 „
100	(CA)	13·6 mm.	20·8 mm.	32·9 mm.
	(CP)	11·5 „	15·0 „	29·3 „

From the above table, it will be seen that the optimum relative humidity for growth of both the strains (CA) and (CP) is 100 per cent. and that the fungus tolerates a wide range of relative humidity from 47-100.

(*v*) ***Temperature relationships.***—The temperature relationships study of the two strains was made on Coons' agar, Richards' solution agar and Prune juice agar. No growth occurred at 5·5°C. and 37·5° in all media. On Richards' solution agar growth took place even at 37·5°C. Both the strains showed optimum growth at 27·5°C. in all media tried.

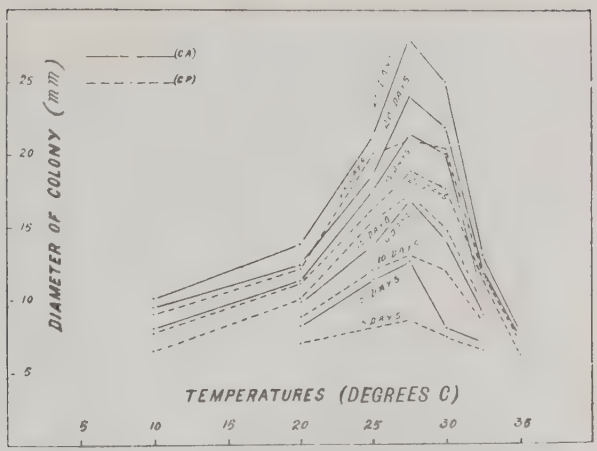

Fig. 1. Growth of *C. indica*, strains (C A) and (C P) on Coons' agar at various temperatures.

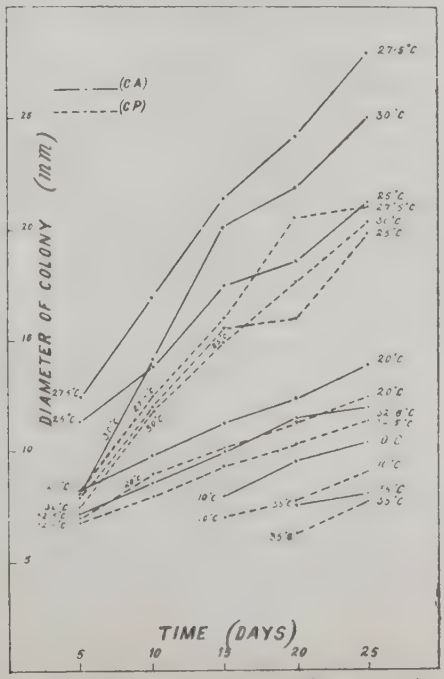

Fig. 2. Temperature relationship of *C. indica*, n. sp. strains (C A) and (C P) on Coons' agar.

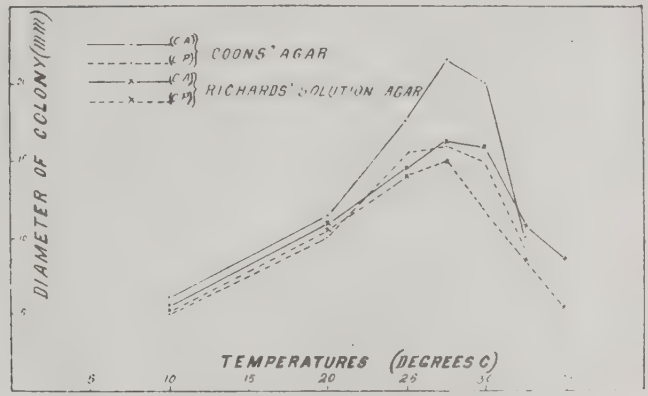

Fig. 3. Fifteen days' growth of *C. indica*, n. sp., strains (C A) and (C P) at various temperatures on Coons' agar and Richards'solution agar.

Figs. 1 and 3 illustrate (*a*) the relation between growth rate and temperature during a fixed time period and (*b*) the relation between growth rate and time at certain temperatures. From Figs. 1, 2 and 3 it is seen that the fungus grows well at temperatures from 20°C.– 30°C. Both the strains show best growth between ten and fifteen days. After fifteen days growth slows down. The rate of linear growth of strain (CA) is greater than (CP) under identical conditions of growth.

(*vi*) *Concentrations.*—Both the strains were grown on different concentrations of Coons' solution. Dry weight of the mycelium was determined after a month's growth. The dry weight of the mycelium in both cases decreases either by increasing or by decreasing the concentration of the normal solution, and thus the best growth takes place at normal concentration of the medium. Table III shows the relative dry weight of both the strains.

TABLE III.

Strains	10 *N*	5 *N*	2 *N*	*N*	*N*/2	*N*/5	*N*/10	*N*/20	*N*/50	*N*/100
	grm.	grm.	grm.	grm.	grm.	grm.	grm.	grm.	grm.	grm.
(CA)	·0246	·0267	·0277	·0345	·0134	·0103	·0015	·0014	·0031	·0009
(CP)	·0179	·0190	·0210	·0220	·0178	·0167	·0149	·0144	·0140	·009

The size of the floating colonies their number and intensity of colour reduce with the lowering of the concentrations. This reduction in the number of floating colonies also takes place with the increase in concentration, at 10 *N* only one big floating colony is present. From *N*/5—*N*/100 submerged portion of floating colonies is not as usual bluish green but pure white. At concentrations above normal, *i.e.*, 10 *N*, 5 *N*, 2 *N*, as well as in *N*, the dry weight of the mycelium of strain (CP) is less than (CA), while at concentrations below normal, *i.e.*, *N*/2, *N*/5, *N*/10, *N*/20, *N*/50 and *N*/100 reverse is the case.

(*vii*) *Importance of different constituents of Coons' solution.*—In order to determine the importance of different constituents of a synthetic solution on growth of (CA) and (CP) flasks of Coons' solution containing 50 c. c. of the medium with one constituent left out were inoculated and kept at 31°C. and dry weight of the mycelium determined after sixty-one days and are given in Table IV.

TABLE IV.

Strains	AVERAGE DRY WEIGHT OF THE MYCELIUM				
	Normal	No $MgSO_4$	No asparagin	No KH_2PO_4	No maltose
(CA)	·0373 grm.	·0200 grm.	·0097 grm.	·0300 grm.	·0002 grm.
(CP)	·0538 ,,	·0205 ,,	·0048 ,,	·0508 ,,	·0007 ,,

From the above table it will be seen that the constituents of Coons' solution in order of their importance can be arranged in the following order, *viz.* maltose, asparagin, magnesium sulphate and potassium acid phosphate. The importance of maltose is greatest since in cultures with no maltose the growth is very poor and the colonies are white in colour. Acid phosphate is of least importance. Except in Coons' solution with no asparagin, the growth of strain (CP) is greater than (CA).

(B) MICROSCOPIC CHARACTERS.

(*i*) *Characteristic of mycelium.*

Aerial mycelium of (CA) is at first hyaline, straight with septa at long intervals, while that of (CP) is usually smoky brown; in older cultures it becomes slightly

yellowish and highly tortuous with abundant chlamydospores in the case of (CA) while in (CP) it becomes more highly torulated with abundant chlamydospores than in strain (CA).

Submerged mycelium of (CP) is thicker than the aerial mycelium and greenish grey with septa at short intervals. In older cultures it becomes dark brown, highly tortuous and torulated. Chlamydospores are found in all media tried except plain agar. Due to the abundance of chlamydospores the submerged mycelium of (CP) is much more highly tortuous than that of (CA) and present even on plain agar.

(ii) Size and septation of spores as influenced by different factors.

(a) Cultural medium.—To see the effect of different media on size and septation of spores both the strains were cultivated on a number of media and the range of length, width and septation of spores were determined at 25°C. on Coons' agar, Prune juice agar, *Cajanus indicus* stems, *C. indicus* leaf decoction agar and wheat straw. Very elongated and thin conidia were produced on *Cajanus indicus* stem and *Cajanus indicus* leaf decoction the proximal end of the spores is small and narrow while the distal end is thin and long (Plate XXII, figs. 23-27). The intensity of sporulation of the two strains is given in Table V.

TABLE V.

Strains	Coons' agar	*Cajanus indicus* leaf decoction agar	Wheat straw	Prune juice agar	*Cajanus indicus* stem
(CP)	XXX	XXXX	X	XXX	XX
(CA)	—	X	—	—	XX

— = no sporulation ; X= slight ; XX= good ; XXX= very good ; XXXX= best.

From Table V it will be noticed that strain (CA) does not form spores on Coons agar, wheat straw and Prune juice agar. A comparison of spore measurements of both the strains on *Cajanus indicus* leaf decoction agar and stem shows some difference in spore length, though they are alike in shape of the spore, width and septation. The size and the septation of spores of strain (CP) is greatest on Prune juice agar and least on Coons' agar while on these medium the strain (CA) does not sporulate.

TABLE VI.

Strains	Media	Length (μ)				Width (μ)		Septation		Septation mode
		Range	Average	S. D.	C. V.	Range	Average	Range	Average	
(CP)	Coon's agar	23·4-136	61·1±1·5	23·58	37	1·7-3·4	3·2	1-10	4	3
(CP)	Wheat straw	20·4-187	70·3±1·1	15·0	21·3	1·7-3·4	3·2	1-12	5	4
(CP)	Prune juice agar.	10·2-278·8	73·4± ·9	13·8	10·8	1·7-3·4	3·3	0-16	6·3	6
(CP)	*Cajanus indicus* stem.	13·6-170	64 ± ·67	10·0	15·6	3·4-5·1	3·2	0-11	4·8	5
(CA)		10·2-183·6	67 ± ·8	12·0	17·9	3·4-5·1	3·2	0-11	4·8	5
(CP)	*Cajanus indicus* leaf decoction agar	12 -148·6	68·5±1·1	15·0	21·8	1·7-3·4	3·4	1-11	5·4	4
(CA)		17·0-156·4	72·5±1·1	17·0	23·2	1·7-3·4	3·5	0-13	5·6	4

(*b*) *Temperature.*—The effect of temperature on size and septation of spores was determined only for strain (CP) on Coons' agar and the data are given in Table VII. Twenty-five days old culture was used in each case (Plate XXII. figs. 28-36).

TABLE VII.

Temperatures °C.	Length (μ)				Width (μ)		Septation		Septation mode
	Range	Average	S. D.	C. V.	Range	Average	Range	Average	
10	No sporulation								
20	20·4-173·4	61·8±1·8	28	41	3·4-5·1	3·5	1-12	4	4
25	23·4-136	61·15±1·5	23·5	37	2·5-3·4	3·4	1-10	4	3
27·5	20·4-129·2	58·65±1·4	20·9	35	2·5-3·4	3·4	1-8	3·9	4
30	20·4-102·8	54·7± ·9	13·8	20	2·5-3·4	3·4	1-7	4	3
32·5	6·8-78·2	36·4±1·2	17·7	48	2·5-3·4	3·2	1-5	2·4	1
35	6·8-68	32·8±1·8	19·0	59	1·7-3·4	2·1	0-3	1·3	1

From Table VII it will be seen that the size and septation of spores is greatest between 20 and 25°C. At 32·5°C., 35°C., the spores formed are smaller in size and with few septa. Sporulation is best at 20°C.

(iii) Sclerotial formation.

Sclerotia like bodies are formed in very old cultures. They are abundantly formed on wheat straw and *Cajanus indicus* stem. These are formed by the irregular divisions of the cells of the mycelium. Often these are more or less rounded bodies of dark brown colour.

(iv) Chlamydospores.

These are found at all temperatures. In strain (CP) these are dark brown with thick walls while in strain (CA) they are greenish brown and thin walled rounded bodies which give the mycelium a beaded appearance (Plate XXII, figs. 37-40).

V. Hydrogen-ion concentration.

Modified Richards' solution of Karrer and Webb [1920] was adjusted to various hydrogen-ion concentrations and four flasks were prepared for each pH value. One of these was put as control while the other three were inoculated. All the flasks were incubated at 27·5°C. for 79 days. After that period the pH values of the filtrates as well as of the control together with the dry weight of the mycelium were determined.

Experimental results and conclusion.—The data are presented in Table VIII and the growth is represented graphically in Fig. 4. Dry weight of the mycelium represents the average dry weight of the mycelia from the three flasks. Growth starts abruptly between pH 2·5 and 2·9, there being no growth at pH 1·7, 2·1 and 2·5, while at pH 2·9 there is fair amount of growth. The growth is uniform over a range of pH 2·9—7·1. Maximum growth occurs at pH 6·7. After that there is a sudden fall up to pH 7·1, beyond which no growth takes place. On the acid side of neutrality no growth took place at pH 1·7, 2·1 and 2·5, while on the alkaline side of neutrality no growth at pH 7·7 and beyond was observed. Only one maximum is obtained and that is at pH 6·7 on the acid side of neutrality. Thus pH 1·7, 2·1, 2·5, 7·7, 8·1 and 9·1 should be regarded as toxic hydrogen and hydroxyl ions, for no growth of the fungus occurred at these pH values.

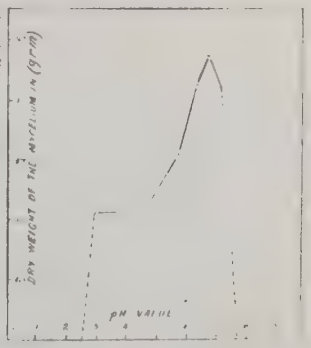

Fig. 4. Dry weight of the mycelium of *C. indica*, strains (C P) in 79 days at 27·5°C. at various hydrogen-ion concentrations.

TABLE VIII.

The growth of (CP) and the changes in reaction induced by growth in modified Richards' solution at different hydrogen-ion concentrations at 27·5°C. in darkness after 79 days.

H-ion concentration			Average dry weight of the mycelium
Initial	Final		
	Control	Inoculated	
1·7	1·7	1·7	No growth
2·1	2·1	2·1	,, ,,
2·5	2·5	2·5	,, ,,
2·9	2·9	2·9	·0556 grm.
4·6	4·1	2·9	·0558 ,,
5·7	5·3	2·9	·0785 ,,
6·3	6·0	2·9	·1100 ,,
6·7	6·2	2·9	·1145 ,,
7·1	6·5	2·9	·1031 ,,
7·7	7·3	7·3	No growth
8·1	7·8	7·8	,, ,,
9·1	8·8	8·8	,, ,,

The fungus during its growth on the modified Richards' solution produced marked changes in the reaction of the medium. The changes in pH value that the control undergoes during the period are shown in Table VIII. It is seen from the table that there has been very little change in pH values of the controls on the acid side of neutrality, while in those on the alkaline side there is remarkable shift in pH values. The pH 1·7, 2·1 and 2·5 remained constant after 79 days, while pH 7·1 7·7, 8·1 and 9·1 shifted to pH 6·5, 7·3, 7·8 and 8·8 respectively. The pH 2·9, 4·6, 5·7, 6·3, 6·7, 7·1 of the inoculated flasks all shifted to a constant pH 2·9, which therefore represents the acidity of the medium on which the fungus grew well. Similar results were obtained on *C. dolichi* by Singh [1933]. In certain vegetable decoctions the change has been reported to be on the alkaline side of neutrality.

The question as to how the fungus passes its dormant period on the surface of Pusa soil which has got a pH value as high as 8·2 while the fungus can not grow beyond pH 7·1 could not be accounted for in view of the fact that the limiting hydrogen-ion concentration is the same in the soil as in the culture solution. But as so many chemical reactions are constantly going on in the soil, it is no wonder that the dormant mycelium could be able to save itself from being totally destroyed and with the return of favourable conditions gives rise to a new crop of conidia.

A study of the growth of the fungus on modified Richards' solution of pH 4·5 was carried out, and the records in the shift of pH of the inoculated and the control were made once after each fifteen days' interval up to sixty days, as given in Table IX below.

TABLE IX.

Days	pH control	Average pH of inoculated	Average dry weight of the mycelium
15	4·5	3·8	·0472 grm.
30	4·3	3·3	·0650 „
45	4·3	2·9	·0739 „
60	4·3	2·9	·0884 „

From Table IX it is seen that there is no change in pH value of the control after 15 days, but the pH of the inoculated is changed from pH 4·5 to 3·8. In 60 days the acidity of the control is increased from pH 4·5 to 4·3, while that of the inoculated ones from 4·5 to 2·9. pH remains constant between 45 and 60 days. The greatest increase in the dry weight of the mycelium takes place between fifteen and thirty days.

VI. Diagnosis of the new species.

There are two species of Cercospora known on *Cajanus indicus*, viz., *Cercospora cajani* (P. Henn) and *Cercospora instabilis* Rangel. Rangel has changed *Cercospora cajani* (Henn) into *Vellosiella cajani* (P. Henn) Rangel. The two strains of *Cercospora* under study, one isolated from Allahabad (CA) and the other from Pusa (CP) are, however, quite different to the two species mentioned above. A comparison of the two strains with the other known species is given below in Table X.

TABLE X.

	Vellosiella cajani (P. Henn) Rangel or *Cercospora cajani* (Henn)	*Cercospora instabilis* (Rangel)	*Cercospora* sp. from Allahabad and Pusa (CA) and (CP)
Symptoms . .	Round spots of a chestnut brown colour with dark brown margin from 2-3 cm. in diameter, on both sides of the leaves. Spots irregular, scattered or sometimes grouped together tufted on the upper surface.	Small angular spots of a dark brown colour with a dark red margin on both sides of the leaves, on branches and fruits also. Spots scattered and aggregated and tufted on both surfaces.	Small irregular spots of a dark brown colour with light brown margin on undersurface of leaf, but in severe cases on both sides. It first appears on the undersurface of the leaves. Spots usually separate, but often several coalesce forming large diseased areas as large as 15 mm. × 5 mm. These spots are tufted on the undersurface of the leaves. Infection of petiole and stem also occurs but never of fruits.
Conidiophores . .	Arising through stoma in clusters, tips bent, septate, pale brown, cylindrical 4-6μ in diameter.	Mostly erect or twisted, abruptly bent like knee joint, septate, of sooty colour, stromata minute protruding 50-80μ Ψ 4-6μ mostly erect or curved. Stromata 20-30μ Ψ 1·5-3μ.	Branched, branching alternate mostly bent arising through stoma in clusters. Stromata very prominent found in the air spaces and sometimes through rarely protruding outside the leaf. Light brown while young dark brown when mature with prominent geniculations on all sides. 28·0μ—168·0μ Ψ 3·4—7·0μ. Septa 2-13.
Conidia . . .	Apically attached bud shaped sometimes forming a part of the conidiophore oblong, sometimes obtuse straight or slightly curved. One septate rarely 2-3 septate, non-constricted, sub-hyaline to pale brown in colour 20-30μ Ψ 4·6μ media (20-24μ Ψ 5·6μ).	Club shaped or vermiform, multiseptate, bent hyaline. 80-200μ Ψ 2·5-4μ.	Hyaline or slightly greenish yellow multiseptate, abruptly obclavate sometimes vermiform with indistinct scars less than 2μ in diameter. Constricted near septa. Strain (CP) (6·8μ—129μ) Ψ (3·4μ—5·1μ). Septation 0 9. Average (38·7μ Ψ 4·2μ). Septation mode 2. Strain (CA) (6·8μ—108μ) Ψ (3·4μ—5·1μ). Septation 0 9. Average 36·8μ Ψ 4·2μ. Septation mode 2.

From the above table it is seen that both the Indian strains are quite different to those previously known and recorded on *Cajanus indicus*. The Indian strains of *Cercospora* further do not agree with any of the *Cercospora* known on any other pulse. Therefore the author considers it to be a new species consisting of two strains (CA) and (CP) which differ from each other only in cultural characters which are of minor importance as regards determination of species as previously mentioned in the text and the name *Cercospora indica* is proposed.

The diagnosis is as follows :

Cercospora indica n. sp., spots minute, irregular, 1-2 mm. in diameter scattered or sometimes aggregated, dark brown with light brown margin, tufted mostly on the under surface in advanced cases on both surfaces. Petiole and stem are also infected. Conidiophores, branched, branching alternate, mostly bent arising from stoma in clusters light brown to dark brown with prominent giniculations 28μ-168μ Ψ 3·4μ-7μ in diameter, 2-13 septa. Stromata prominent in air spaces rarely protruding out of the stoma. Conidia hyaline, or slightly greenish yellow multiseptate, abruptly obclavate sometimes vermiform with indistinct scars less than 2μ in diameter, often constricted near septa 6·8μ 129μ Ψ 3μ-5μ in diameter average by 37μ Ψ 4μ. Septation 0-9 with mode at 2.

Habitat. On leaves, petioles and stems of *Cajanus indicus* Spreng. Allahabad and Pusa.

Type specimen. Deposited in Pusa herbarium and Allahabad University herbarium.

VII. Summary.

1. The leaf-spot disease of *Cajanus indicus* caused by two strains of *Cercospora* is of very common occurrence. The symptoms of the disease and the morphology of the fungus are described.

2. Artificial infection takes place through spores only. Infection of other pulses does not take place. Between 20°C. and 25°C. infection occurs readily.

3. A comparison of cultural character of the two strains on a number of media shows remarkable differences.

4. Growth of strain (CA) is greater than (CP) in all media. Using Coons' agar it was found in case of both the strains that growth of the fungus increases with the increase in the amount of media ; it is more in alternate light and darkness less in continuous darkness and least in continuous light. It grows through a wide range of relative humidity from 47-100 per cent. Best growth of both strains takes place at 100 per cent. humidity.

5. Optimum temperature for growth for both the strains is 27·5°C. No growth at 5·5°C. and 37·5°C. except on Richards' solution agar where the growth also takes place at 37·5°C.

6 Growth of both the strains is retarded both by diluting and concentrating Coons solution. Maltose is the most important constituent of Coons' solution for growth of both the strains asparagin, magnesium sulphate potassium acid phosphate being of less importance.

7 Best sporulation takes place at 20°C. 25 C. No formation of spores at 10°C. or below. Length and septation of spores is greatest at 20 C. 25 C. and decreases with the increase or decrease of temperature.

8 The fungus renders the medium on which it grows acid and tolerates a wide range of pH 2·9-7·1. Optimum growth is at pH 6·7. No growth at pH 1·7, 2·1, 2·5, 7·7, 8·1 and 9·1 has been observed.

9 Both the strains have been found to belong to one species which has hitherto not been described and is named *C. indica* the diagnosis of which is given

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IX. Explanation of Plates.

PLATE XXI.

(The figures are reduced to 2/3.)

Figs. 1 and 2.—Early stages in the infection of leaf. Undersurface. (· 1½).
Fig. 3.—Later stage of the same. (· 1½).
Fig. 4.—Advanced stage in the infection of leaf. Upper surface. (1½).
Figs. 5 and 6.—Enlarged young and old spots of leaf. (· 8).
Fig. 7.—A portion of petiole showing infection spots. (1½).
Fig. 8.—A portion of stem showing infection spot. (1½).

PLATE XXII.

(The figures are reduced to 2/3.)

Fig. 1.—A portion of T. S. of leaf showing emergence of conidiophores through a stoma. (· 800)
Fig. 2.—A portion of T. S. of leaf showing a fascile of conidiophores bearing conidia. (· 800).

Fig. 3.—A fascile of conidiophores from host. (×800).

Figs. 4, 5 and 6.—Conidiophores from host. (× 1,850).

Figs. 7, 8, 9, 10, 11, 12, 13, 14 and 15.—Typical conidia of Strain (CP) from host. (× 1,850).

Figs. 16, 17, 18 and 19.—Typical conidia of the strain (CA) from host. (× 1,850)

Figs. 20 and 21.—Germinating conidia from host. (× 1,850).

Fig. 22.—Two conidia fusing from host. (× 1,850).

Figs. 23 and 26.—Typical conidia from sterilized *rahar* stem at 20°C., 25 days old culture. (× 1,850)

Figs. 24, 25 and 27.—Typical conidia from *rahar* leaf decoction agar at 30°C., twenty-five days old culture. (× 1,850).

Figs. 28, 29, 30, 31, 32 and 33.—Typical conidia from Coons' agar at 27·5°C., 25 days old culture (× 1,850).

Figs. 34 and 35.—Conidiophores with conidia attached from Coons' agar at 32·5°C., one month old cultures. (× 1,850).

Fig. 36.—Conidiophore with a conidia attached on oat meal agar at 30°C., one month old culture (× 1,850).

Figs. 37 to 40.—Stages in the formation of chlamydosphores, from Coons' agar at 27·5 C. (× 800).

PLATE XXIII.

Fig. 1.—Twenty-five days old culture of *C. indica*, strain (CA) on Coons' agar at 27·5°C.

Fig. 2.—Twenty-five days old culture of *C. indica*, strain (CP) on Coons' agar at 27·5°C.

Fig. 3.—107 days old culture of *C. indica*, strain (CA) on rice meal agar at 27·5 C. showing fan sectors.

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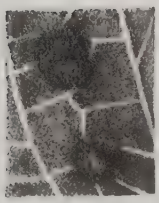

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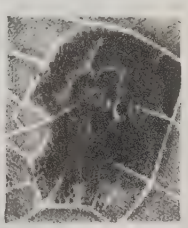

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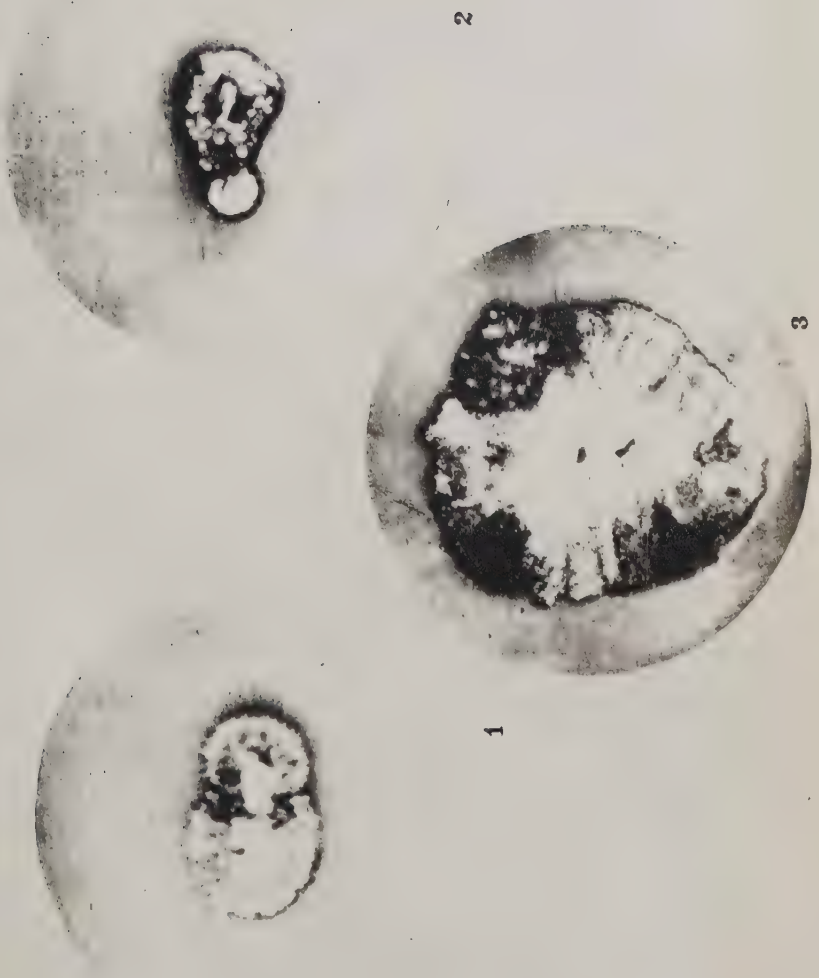
(*For explanation see page* 359.)

PLATE XXII.

(*For explanation see page 359.*)



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